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COMPREHENSIVE RESEARCH ON RICE
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PROJECT TITLE: RB-3: Rice Genetics and Germplasm Development

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OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

1. **Rice Genetic Resources.** Objectives are to maintain and evaluate a diverse set of rice varieties and wild species, import useful new germplasm and introduce useful traits into California varieties.

   1995 Experiments:
   1. Evaluation of introduced germplasm, hybridization, and breeding nurseries.
   2. Identification of new RAPD primers to differentiate California rice cultivars.
   3. Evaluation of AFLP and microsatellite markers for genetic studies in japonica rice.

2. **Identification of useful genes.** The main strategy is to use DNA markers to "tag" important genes. The markers are linked closely to the genes of interest, and their chromosomal location is known or can be easily determined.

   1995 Experiments:
   1. Seedling vigor.
   2. Submergence tolerance.
   3. Stem rot resistance.

4. Cold tolerance.
5. Water weevil tolerance.

3. **Hybrid rice.** Hybrid rice production has been spreading in Asia, and interest has grown in the US. Genetic mechanisms have now been developed for commercial hybrid rice production. Whether these will be viable in California depends on the cost of seed and the availability of a hybrid with sufficient yield advantage and acceptable grain quality. Our research focuses on genetic mechanisms of seed production.

1995 Experiments:
1. Transfer of cytoplasmic male sterility (cms) and restorer genes into California cultivars.
2. Transfer of wide compatibility genes into California cultivars.
3. Development of photoperiod-sensitive genic male sterility (PGMS) for hybrid rice seed production.

SUMMARY OF 1995 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE

1. **Rice genetic resources**

Evaluation of introduced germplasm breeding nurseries. About 250 new introductions from the US germplasm collection were grown and evaluated at Davis in 1995. Several entries were selected for further evaluation. Over 100 germplasm accessions from a previous study on genetic diversity in japonica rice were also grown. The Regional Uniform Nursery (RUN) from the southern US was grown. Additional backcrosses were made to M-202 to introduce genes from *O. nivara* into *O. sativa.* We also grew 1,900 pedigree lines and evaluated 14 F_2 populations.

RAPD primers and California cultivars. We have been using RAPD (random amplified polymorphic DNA) markers to "fingerprint" California rice cultivars. In 1995 we screened additional primers and identified several that are effective in differentiating California cultivars (Table 1).

AFLP and microsatellite markers. We have been studying the application of two new types of molecular markers for genetic studies in japonica rice. Amplified fragment length polymorphism (AFLP) is a technique to generate a large number of markers that can be used for mapping or tagging important genes. We studied the variability for these types of markers with a sample of 14 rice accessions. The percent polymorphism for AFLP markers was lower than for RFLP or RAPD markers (around 30% of the markers in the sample were polymorphic), but the large number of markers assayed compensated for this lower polymorphism. The 17 AFLP primer combinations were able to detect 529 bands, of which 147 were polymorphic in this sample. These 147 markers were used to characterize the sample by cluster analysis. The results of the classification agreed well with a previous analysis using 43 RAPD markers (Fig. 1), and showed clearly that the US cultivars could be classified either as tropical (long grain) or temperate (medium grain) japonicas. We mapped 50 AFLP markers on the rice chromosomes using a cross between Black Gora (indica) and Labelle (japonica). These genetic markers were distributed throughout the rice genome (Fig. 2), indicating that AFLP markers should be useful in gene mapping of rice. We estimate that over 30,000 loci could be assayed in 2-3 months with this system.

Microsatellite markers are different from AFLP in that only a single marker or locus is assayed per reaction. With this type of marker, two primers amplify a specific chromosomal segment that is known to be highly variable due to repeats of from 2 to 5 DNA base pairs. The advantage of these loci is that they are much more polymorphic than other types of markers, and usually several alleles can be detected per locus. We used microsatellite markers in the sample of rice cultivars described above. The results showed that these markers were polymorphic even within japonica cultivars. However, closely related cultivars such as the California medium grains could not be distinguished with all 14 markers tested. Even so, with correct choice of parents it may be possible to use microsatellite markers for mapping genes within japonica rice crosses. The main limitation now is that very few of these markers have been mapped, and it is quite expensive and time consuming to identify the large number of loci needed for gene mapping.

2. Identification of useful genes

Seedling vigor. Last year we identified quantitative trait loci (QTL) controlling seedling vigor traits in the cross Labelle X Black Gora. Unfortunately, QTL for shoot length could not be identified in the high-vigor parent Black Gora. We therefore attempted to identify QTL for seedling vigor traits in a cross between two japonica cultivars: Italica Livorno (high vigor) and Labelle (low vigor). Because RFLP polymorphism was very low in this cross we used RAPD markers. This resulted in the first molecular map to be constructed from a cross between two japonica cultivars (Fig. 3). Interestingly, our analysis indicated that polymorphism was much higher for chromosomes 10 and 11 compared to that observed in indica X japonica crosses or crosses involving wild species (Fig. 4). In contrast, polymorphism was much lower for chromosomes 1 and 2. These results indicate that tropical and temperate japonica cultivars show considerable diversity for chromosomes 10 & 11 compared to the normal indica-japonica diversity.

Seedling vigor QTL were identified in this cross that were different from those identified in the indica X japonica cross (Fig. 3). For the first time, we were able to identify a shoot-length QTL from a high-vigor parent (Italica Livorno). This QTL, linked to the RAPD marker $OPAD13_{720}$ on chromosome 3, controlled about 18% of the variation in this cross.

Submergence tolerance. Submergence tolerance may be a useful trait for rice varieties if deep water is used at seeding as a method of weed control. Strong sources of submergence tolerance have been identified in indica rice cultivars. We were successful in identifying a major gene controlling this trait in rice, the first such gene ever identified (Fig. 5). This gene, located on rice chromosome 9, has been designated *Sub1*. We are currently transferring this gene into M-202 background. We are also identifying markers that are more closely linked to the gene so they can be used in marker assisted selection (MAS).

Stem rot resistance. Recombinant inbred populations for studying the genetics of stem rot resistance were developed by Jeff Oster from segregating populations supplied by S. T. Tseng. Resistant and susceptible F_6 lines were identified in each of four crosses. We are currently screening the parents (*O. rufipogon*, 87-Y-550, and susceptible parents) to identify polymorphisms that may be linked to the stem rot resistant genes. We have screened over 700 RAPD primers, but have not detected positive polymorphisms. We are beginning to use AFLP primers to increase the number of loci we can assay in an attempt to locate the gene(s) conferring stem rot resistance in 87-Y-550.

Cold tolerance. In 1995, we subjected the F_2 population M-202/IR50 to low temperature during the booting stage in an attempt to identify genes conferring cold tolerance. These plants were subjected to a temperature treatment of 15°C in the growth chamber. Unfortunately, the results were not consistent, and the level of stress was too low. This was most likely a result of warmer than expected plant temperatures due to proximity to the lights. Screening will be attempted again using a lower temperature.

Water weevil tolerance. The populations for the study of the genetics of water weevil tolerance are still being advanced and will not be ready for screening for 1-2 years.

3. **Hybrid rice**

Cytoplasmic male sterility (cms). Research focused on developing California-based germplasm with the genetic mechanisms necessary for hybrid rice production. We have completed transfer of the WA source of cytoplasm into several California cultivars (Table 3). In addition, we are transferring cms from the Chinsurah Boro source and also from an unknown source (obtained by Kent McKenzie). The main limitation to exploiting these lines is lack of restorer genes in japonica rice cultivars. Crosses with most japonica cultivars results in sterile F_1s (i.e. they are maintainers). We have been introducing restorer genes from the indica cultivar IR50R. F_5 lines from these crosses will be grown in Hawaii this winter.

Wide compatibility. In order to obtain fertile hybrids from indica X japonica crosses we are transferring the wide compatibility gene into California medium grain cultivars. The progress on this work is described in the report of a separate project (P. Ronald).

Photoperiod-sensitive genetic male sterility. Last year we reported the selection of 800 new male sterile mutants, including 615 that were spontaneous mutants found in a grower's fields. In 1995 we evaluated these mutants in the field and the greenhouse. The original plants that were spontaneous mutants (615 total) were maintained by vegetative propagation, and the seed harvested from the original male sterile plants was planted in the field. The results of our first year of screening are summarized in Fig. 6. Of the 615 plants originally selected, 547 produced sufficient seed to grow in the field. From these we identified 42 lines that behaved as typical PGMS plants, and another 35 lines that were potential PGMS. In addition, we identified 74 mutants that are candidates for a dominant male sterile mutant. If any of these are truly dominant steriles, this would be a very useful breeding tool, as such mutants are currently not available for rice. It is remarkable that, from the 547 lines evaluated, only 14 were normal (i.e. completely fertile). It thus appears that this approach (i.e. selection of male steriles as spontaneous mutants in growers' fields) is very effective in isolating new male sterile mutants.

PUBLICATIONS OR REPORTS:

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Redoña, E. D., and D. J. Mackill. 1995. Mapping seedling vigor genes in indica and japonica rice with molecular markers. p. 71. *In* Plant Genome III Abstracts. Scherago International Inc., New York.

Xu, K, and D. J. Mackill. 1995. Tagging genes for submergence tolerance in rice. p. 89. *In* Plant Genome III Abstracts. Scherago International Inc., New York.

Colowit, P. M., J. J. Oster, and D. J. Mackill. 1995. Progress in identifying genes conferring stem rot resistance in rice. p. 10, *In* Rice Field Day, 30 Aug 1995, Rice Experiment Station, Biggs, CA.

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Inukai, T., R. J. Nelson, R. S. Zeigler, S. Sarkarung, D. J. Mackill, J. M. Bonman, I. Takamure, and T. Kinoshita. 1994. Allelism of blast resistance genes in near-isogenic lines of rice. Phytopathology 84:1278-1283.

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Mackill, D. J., J. N. Rutger, and C. W. Johnson. 1994. Allelism and pleiotropy of extended upper internode rice mutants. SABRAO J. 26:11-17.

Redoña, E. D., and D. J. Mackill. 1994. Identification and interval mapping of seedling vigor QTL's in rice. Rice Genet. Newsl. 11:124-126.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

In 1995, progress was made in the genetic characterization of US rice cultivars with molecular markers. New RAPD markers were identified that differentiate California cultivars. AFLP markers were evaluated and found to be abundant in rice and randomly distributed throughout the genome. They should be promising markers for tagging genes useful in rice breeding. Initial results with microsatellite markers indicate that, when enough of them have been identified, they will be valuable tools for gene tagging in japonica rice cultivars.

Quantitative trait loci (QTL) were identified for seedling vigor related traits from the japonica cultivar Italica Livorno. This included a shoot-length QTL that appears to be important in seedling vigor. The submergence tolerance gene *Sub1* was identified and mapped on rice chromosome 9. Work continued on stem rot resistance, cold tolerance, and water weevil tolerance.

CMS lines were developed for hybrid rice production. Work continued on the introduction of restorer genes and of wide compatibility into California genetic background. New male sterile mutants were characterized, and some appear to be promising candidates for photoperiod-sensitive genetic male sterility (PGMS). PGMS lines could be a useful component of hybrid rice production, as it would decrease the cost of producing hybrid seed.

Table 1. RAPD primers successful in differentiating California medium grain cultivars based on a single test. (1 = band, 0 = no band).

Primer	M-201	M-202	M-203	M-401
A8			1	0
B18	1	0	1	1
E9	1	0	0	0
F4	1	0	0	0
F14	1	0	1	1
G11-1	0	0	1	1
G11-2	0	0	0	1
K7-1	1	0	1	1
K7-2	0	1	0	0
L18	0	1	0	0
N14-1	0	1	1	1
N14-2	1	0	0	0
N14-3	0	1	0	0
N16	0	1	0	
S8-1	1	0	0	0
S8-2	0	1	1	1
T7-1	1	0	1	1
T7-2	1	1	0	0
T7-3	0	1	0	0

Table 2. Genotypes of microsatellite markers for 14 rice cultivars and breeding lines. Alleles are numbered sequentially beginning with 1 for the largest fragment. The shaded area shows the monomorphism for California medium grain cultivars.

	Microsatellite marker													
Cultivar	R1	R2	R3	R4	R5	R6	R7	R9	R10	R122	R148	R164	R167	R168
L-202	5	1	3	4	1	3	1	1	1	3	1	1	2	4
L-203	5	1	3	2	1	3	1	1	1	3	1	1	1	4
Labelle	5	1	3	4	1	3	2	1	1	3	2	1	2	4
87-Y-550	5	1	3	1,4	1	3	1	1	1	3	1	1,3	1,2	3
Black Gora	4	1	1	5	4	2	3	1	1	1	2	4	2	2
IR40931	6	1	2	3	2	1	3	1	0	3	2	4	2	1
M-103	5	1	4	4	2	3	1	1	1	2	1	3	1	3
M-201	3	1	4	4	2	3	1	1	1	2	1	1	1	3
M-202	3	1	4	4	2	3	1	1	1	2	1	1	1	3
M-203	3	1	4	4	2	3	1	1	1	2	1	1	1	3
M-204	3	1	4	4	2	3	1	1	1	2	1	1	1	3
M-401	3	1	4	4	2	3	1	1	1	2	1	1	1	3
Italica Liv.	2	1	2	4	4	3	2	2	1	2	2	2	1	5
WC 1403	1	1	5	4	3	3	2	2	1	2	2	1	1	3

Table 3. Cytoplasmic male sterile lines developed in California background.

Designation	Davis cross number	Male (Maintainer)	Pedigree
M202A-CB	DX191	M-202	Wu10A/M-202eui//M-202///M-202
M202A-WA	DX193	M-202	IR68289A/M-202*6
M204A-WA1	DX194	M-204	IR58025A/M-204*6
M204A-WA2	DX195	M-204	IR58025A/M-204*3//M-202///M-204*2
L203A-RA	DX197	L-203	CMS-RA/L-203//Michikogane///L-203*3

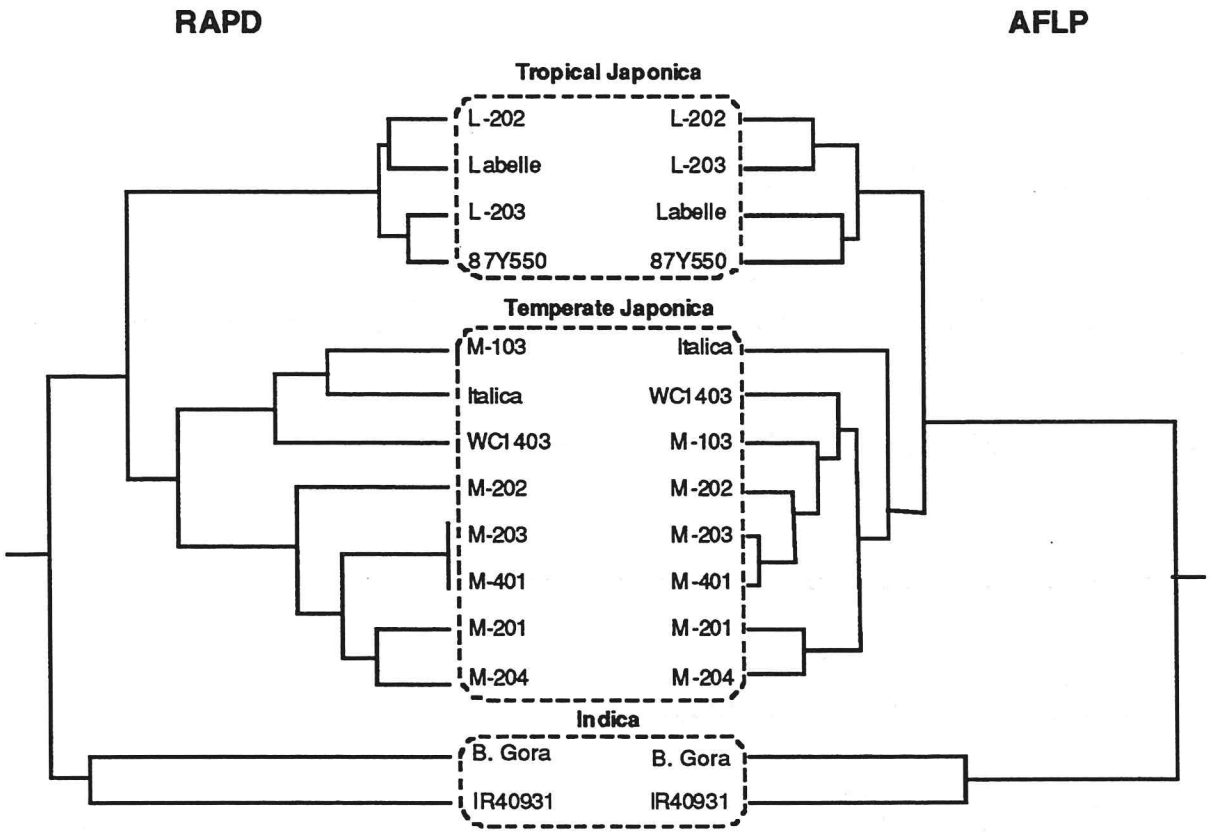

Fig. 1. Cluster analysis of 14 rice cultivars based on RAPD (left) and AFLP (right) markers.

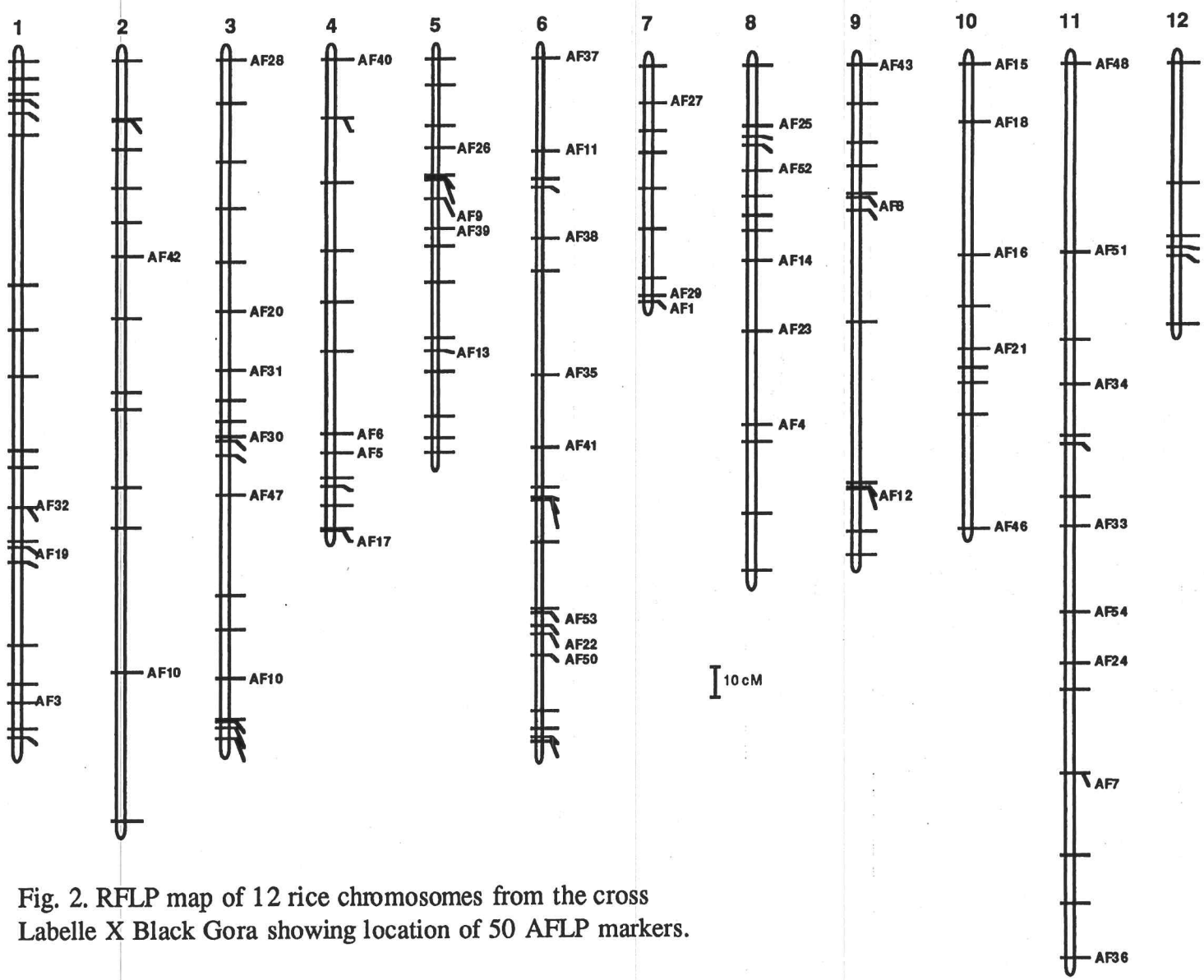

Fig. 2. RFLP map of 12 rice chromosomes from the cross Labelle X Black Gora showing location of 50 AFLP markers.

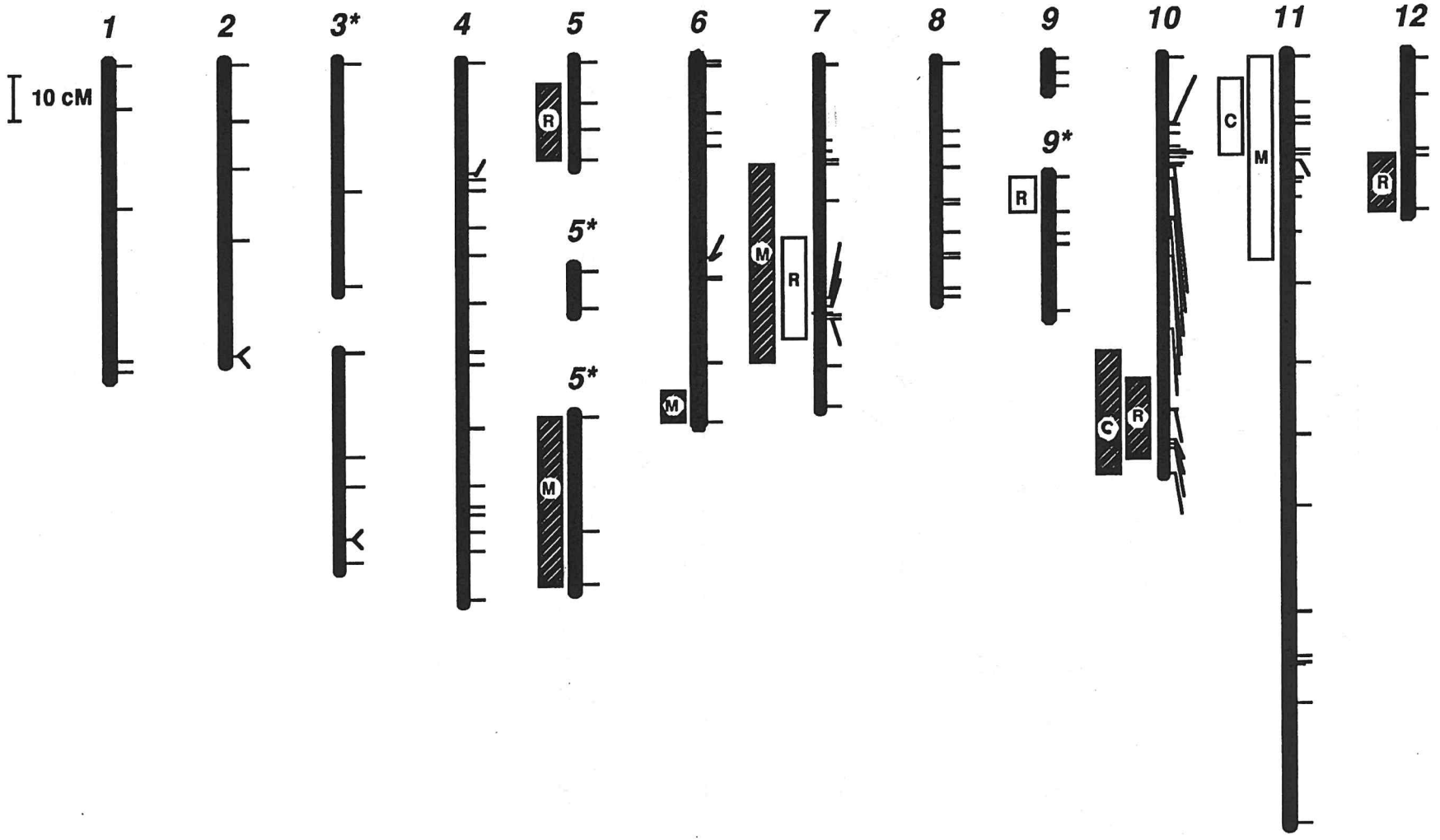

Fig. 3. Japonica rice map (LBL x IL cross) based on RAPDs and RFLPs (shown as horizontal lines) and putative locations of seedling vigor QTLs (R = root, C = coleoptile and m = mesocotyl lengths). Striped and clear bars indicate IL and LBL alleles, respectively, and bar length represents a one-LOD support interval for QTL peak.

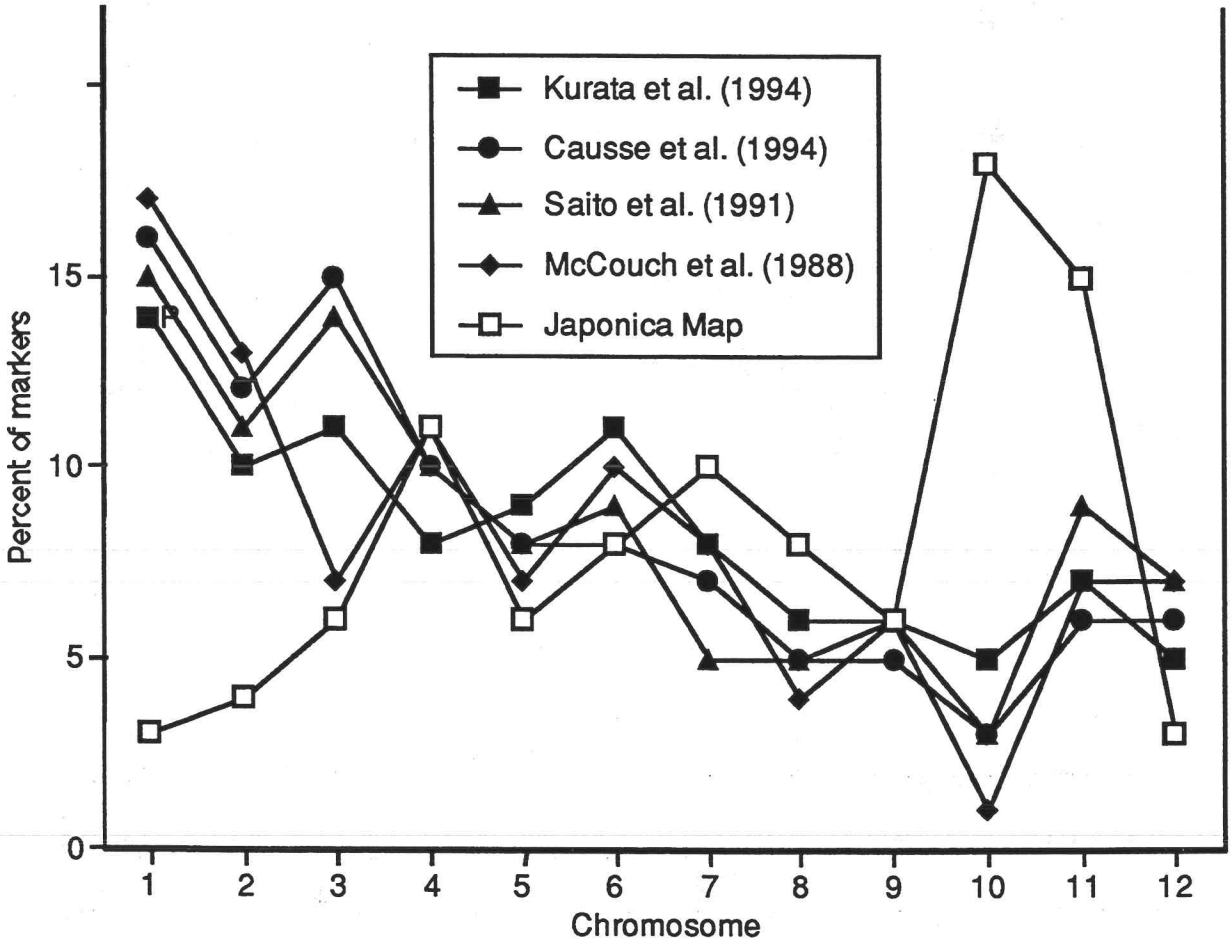

Fig. 4. Comparison of japonica map with other published rice molecular maps based on the percentage of markers mapped per chromosome.

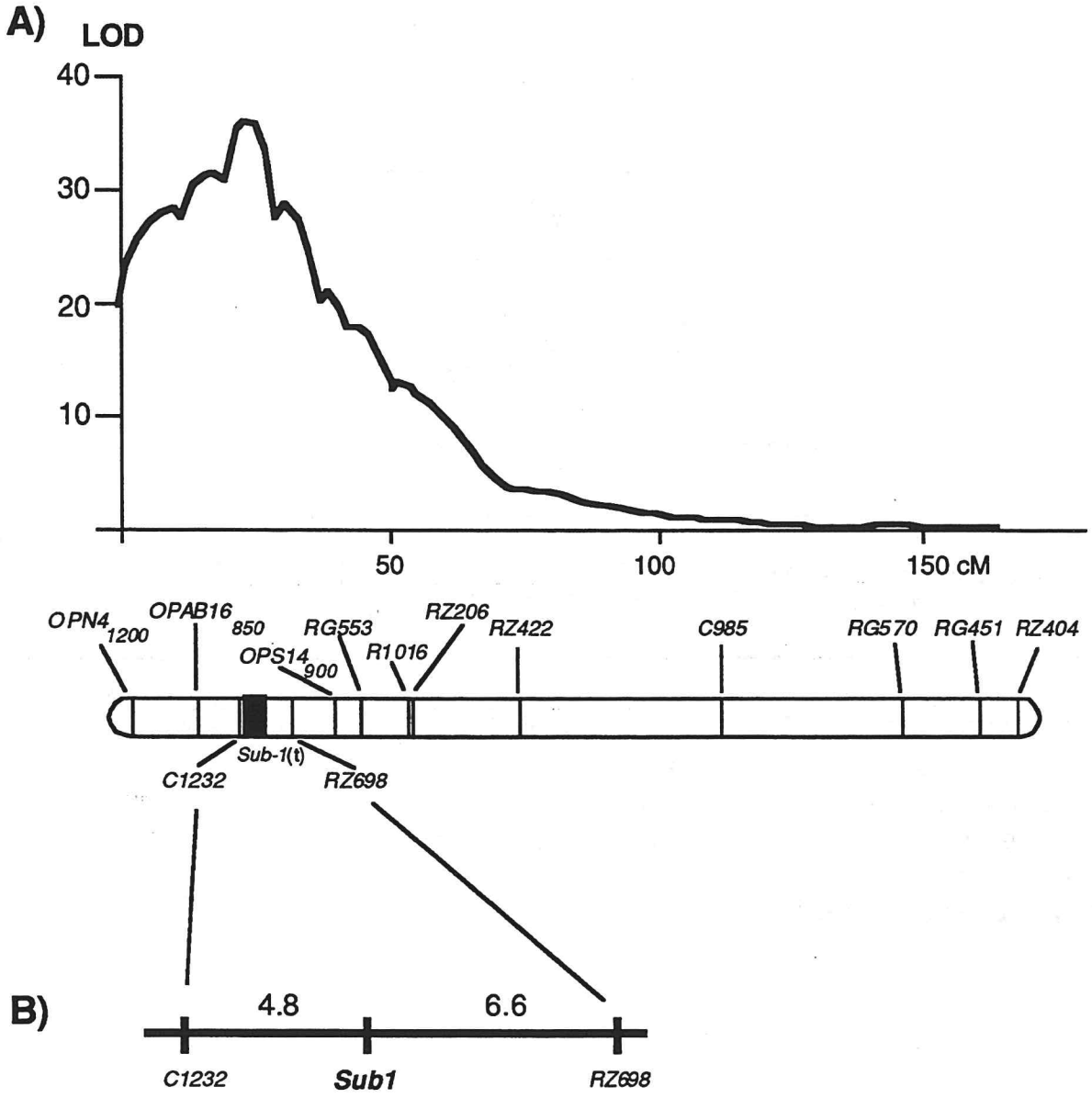

Fig.5 Location of the *Sub1* on the RFLP map of rice chromosome 9. **A)** Location of *Sub1* based on QTL analysis with Mapmaker/QTL computer program. Analysis was based on F_3 mean submergence tolerance scores, and the locus (dark bar) was designated at the position representing a decline of 1 LOD value. **B)** Distance in cM of *Sub1* from the RFLP markers *C1232* and *RZ698* when F_2 plants were classified as tolerant or intolerant based on F_3 plant scores.

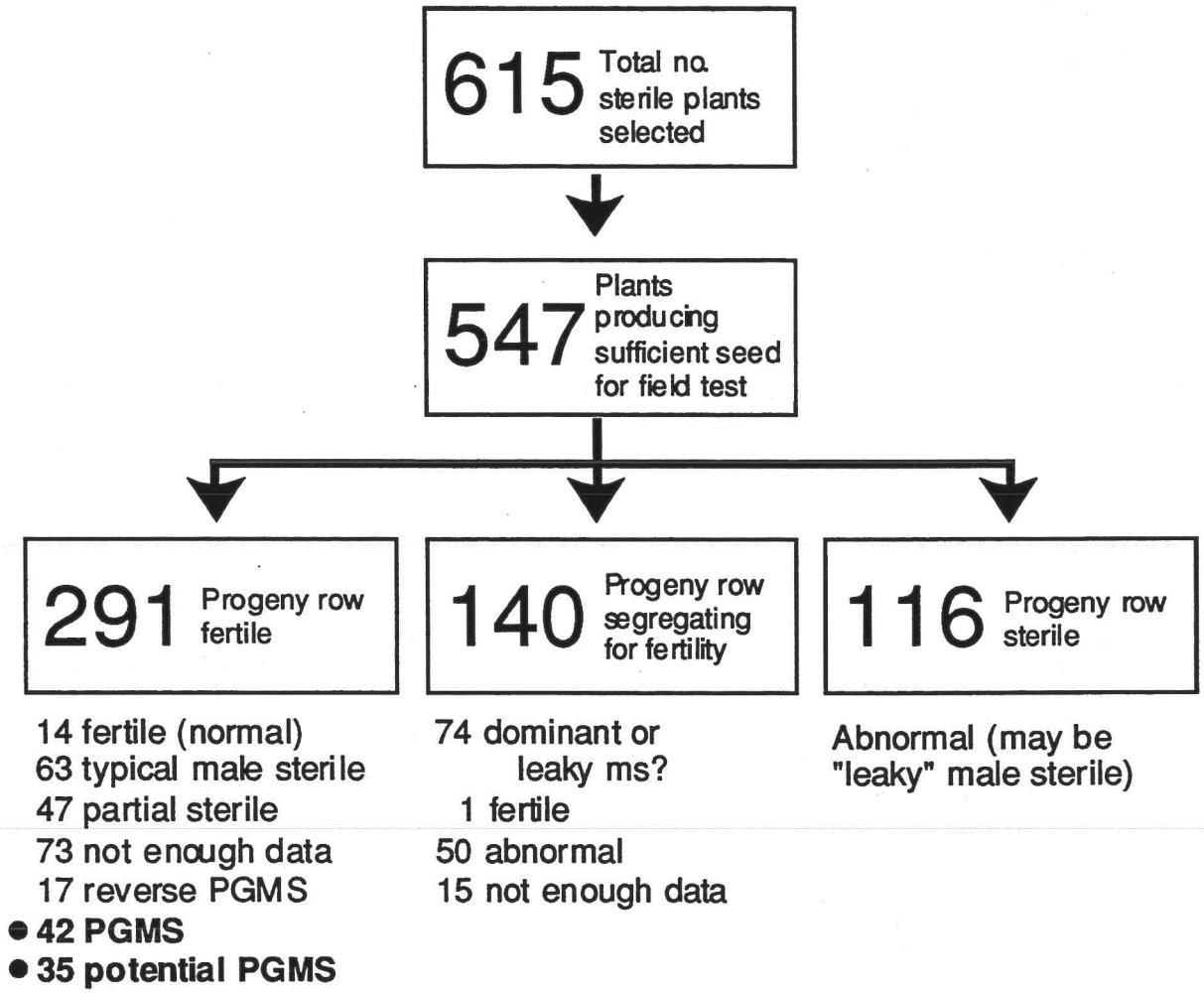

Fig. 6. Screening of putative mutants for sterility behavior. Of the original 615 plants identified, 547 produced sufficient seed for field screening of the progeny. 291 rows were fertile in 1995, which would be expected for true genetic male steriles. Based on the fertility of the original male sterile plants (maintained vegetatively) under long and short day lengths, these lines were further classified into seven groups. Of these, 42 behaved as photoperiod-sensitive genetic male sterile (PGMS) mutants and another 35 were potential PGMS mutants.